Asian Journal of Chemistry; Vol. 23, No. 4 (2011), 1807-1809

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Analysis of Free Phenols from Two Varieties of *Vitis vinifera* in Different Geographical Origins

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(*Received*: 26 August 2010; *Accepted*: 24 December 2010) AJC-9420

Two grape (*Vitis vinifera*) varieties such as *Cabernet sauvignon* and *Cabernet gernischet* collected from two different geographical origins (Penglai of Shandong Province and Changli of Hebei Province) were used for analysis of gallic acid, catechin, epicatechin and epigallocatechin, contents of these compounds were investigated by HPLC (high performance liquid chromatography). The results showed that the phenolic compounds were different in the same variety from different areas ($p < 0.05$)). The content of catechin was the highest among these varities and the contents of other compounds were catechin > epigallocatechin > epicatechin > gallic acid.

Key Words: Varieties, Free phenols, Geographical origin, *Vitis vinifera*.

INTRODUCTION

Phenolic compounds play an important role in wine. They are a group of polyphenolic compounds including phenolic hydroxyl group, which are usually the secondary metabolites in plants. There are five kinds of major phenolic compounds in the ripe grape berries of *Vitis vinifera*[1]: phenolic acids (gallic acid, syringic acid, benzoic acid, *etc*.), flavonols (quercetin, kaempferol, *etc*.), flavanols (catechin, epicatechin, epigallocatechin, *etc*.), flavanonols and anthocyanins. These substances significantly affect the development of wine flavor and the changes of wine colour[2]. Especially, the effects of these substances are more and more obviously with the changing of phenolic compounds in red wine. Among these compounds, anthocyanins are the main resource of the grape colour. The phenolic compounds stabilize the combination of the anthocyanin molecule to increase the colour value[3]. The phenolic compounds are mainly derived from the grape berry. They are related to the colour, sense and taste of the wine[4]. The contents and ratios of the different compounds determine the potential characteristic and flavor of wine, which are both influenced by geographical origins and varieties[5]. Because of these reasons mentioned above, the wine quality is related to the characteristics of geographic origin and the feature of variety. In this study, the differentiation of the geographic origin was investigated in order to obtain some theoretical explanation of the feature of geographic origin. A lot of information of the different phenolic compounds were reported at present, including the ripening of the grape berries of many cultivars, such as *Cabernet sauvignon*, *Melot*, *Tempranillo*, *Syrah*[6-9], *etc*.

EXPERIMENTAL

The varieties of *V. vinifera* were *Cabernet sauvignon* and *Cabernet gernischet*, 5 years old, growing on the vineyard of Penglai of Yantai (Shandong) and Changli of Qinhuangdao (Hebei), where the experimental materials were picked randomly. In this process, the same ripe grapes were selected considering of the sugar-acid ratios.

Sampling and extraction of phenolic compounds: One hundred of grape clusters were randomly collected from the healthy bunches of the whole grapevine on the same day in Penglai and Changli, stored at -20 ºC for later analysis. The upper, middle and underneath fruits were picked by the ratio of 3:2:1, then the phenolic compounds were extracted.

The samples were counted, weighed (2.0 g), then grinding with 200 mL of 65 % (v/v) cold alcohol solution (including 0.5 % sodium metabisulfite, w/v) in the mortar for 0.5 h. After extraction, solutions were collected by the refrigerated centrifuger (10,000r, 20 min) and then the suspension was evaporated under reduced pressure to remove the alcohol. The solutions were defatted by petroleum ether and cleared by a centrifuger (10,000r, 10 min), then the compounds was extracted by ethyl acetate for three times (the ratio of extracts to ethyl acetate was 1:1) and the organic phase was collected, evaporated and dried at 45 ºC. The extracts were dissolved by methanol

and filtered through a 0.45 μm nylon filter and analyzed by HPLC (high performance liquid chromatography)[10].

Standards: Standards of (+)-catechin, (–)-epicatechin, gallic acid and (–)-epigallocatechin were purchased from Sigma (Sigma-Aldrich Co. Ltd., USA), prepared in acetonitrile (100) and the concentration of primary standards were diluted to 1 mg mL^{-1}, respectively.

HPLC quantification: HPLC with a UV-visible detector to analyze the sample extracts. The HPLC system (Waters 996, USA) was equipped with a pump and a PAD detector (210-900 nm), a model autosampler (injection volume of 20 μL). A Shimadzu XB C_{18} analytical column (5 μm, 4.6 mm × 250 mm, Shimadzu Co. Ltd., Japan) and a precolumn with a mobile phase of acetonitrile (B): water (0.1 % acetic acid, w/v) by gradient elution as follows: At 0 min 12 and 88 % B; at 10 min, 12 and 88 % B; at 35 min, 15 and 85 % B. During the processes, the flow rate was of 1 mL min^{-1}, the column temperature was 30 ºC.

RESULTS AND DISCUSSION

HPLC analysis: Four monomers were investigated from the extraction of the frozen grape samples collected from different areas. The relative contents of the compounds analyzed in the experiments are shown in Table-1. It could be concluded that the same cultivar from the different geographical origin had different concentration of the same compounds ($p < 0.05$). For *C. sauvignon*, the ratio of the monomers in the samples of Penglai was less than that from Changli and similar trend was also observed in *C. gernische*.

Extracts from different grape cultivars were analyzed to determine the relative content (Table-1). They were determined at 280 nm and the relative contents were analyzed by t-test (Table-2). From Table-2, it is accounted that the value of p was 0.000 ($p < 0.05$). From the results of this test, there was significantly differences in the four free phenols from the geographic origins, it is suggested that the different geographic origins could affect the quality of the grapes.

Phenolic compounds in the different cultivars from the same geographic origin still possess different contents. For Penglai, the relative content of free phenols from *C. gernischet* was relatively more than that from *C. sauvignon*. The relative content of (–)-epicatechin from *C. sauvignon* was less than that from *C. gernischet*, but the other free phenols possessed the contrary relative contents.

From the table, it could be found that the relative content of (+)-catechin was the highest of all the free phenols detected and the second was (–)-epigallocatechin, then (–)-epicatechin and gallic acid in turn.

The amount of the phenolic compounds existed in the grape berries were mainly decided by the cultivars, seasonal conditions, geographic origins and cultural practices[11]. Penglai lies in the northern of the North coast of Shandong Peninsula, between east longitude 120°35'-121°18' and 37°25' north latitude-37°50', Changli lies in the north of Hebei province, between east longitude 118°45'-119°20' and 39°22' north latitude 39°48'. Different environment formed by different geography symptom greatly affected the growing of the grapes, effective accumulated temperature, solar intensity, edatope and rainfall should be the most important factors in the process of grapevine growing and also affected the quality of the grapes[12]. The same cultivar in the different geography might form different quality, too. The different cultivar in the same area through long time choice and adaptation could form the similar characteristics. As the above table, it could be concluded that the free phenols in two different cultivars such as *C. gernischet* and *C. sauvignon* in the same geography origin were similar to each other, which were in accordance with the literature reported before[13].

Fertile soils of Changli are most suitable for growing of the crops, moisture soil, cinnamonic soil, saline soil and aeolian soil were distributed in local area. However, the region of Penglai is mainly hill mountains, carse, where silty loam and grit soils were formed *i.e.*, the most pH value was 6.0-7.0, the contents of grape acids could be higher by the effects of

TABLE-1
RELATIVE CONTENTS OF THE DIFFERENT GEOGRAPHIC ORIGINS AND CULTIVARS ($\bar{x} \pm s$, n = 3)

Cultivar	Gallic acid relative content (%, w/w)	Catechin relative content (%, w/w)	Epicatechin relative content (%, w/w)	Epigallocatechin relative content (%, w/w)
C. sauvignon (Penglai)	2.85 ± 0.01	24.80 ± 0.15	3.69 ± 0.09	14.03 ± 0.28
C. sauvignon (Changli)	3.42 ± 0.03	27.09 ± 0.21	7.66 ± 0.16	21.30 ± 0.33
Penglai-Changli	-0.57	-2.29	-3.97	-7.27
C. gernischet (Penglai)	3.05 ± 0.04	25.27 ± 0.19	4.73 ± 0.12	15.31 ± 0.22
C. sauvignon (Changli)	3.28 ± 0.02	26.80 ± 0.30	8.28 ± 0.15	20.98 ± 0.27
Penglai-Changli	-0.23	-1.53	-3.55	-5.67

TABLE-2
RESULTS OF FOUR FREE PHENOLS BY t-TEST FROM THE GEOGRAPHIC ORIGINS OF CHANGLI AND PENGLAI

	Geographic origin	Samples	Mean	Degree of freedom	P (2-tailed)
Gallic acid	Changli	6	2.95 ± 0.11	10	0.000
	Penglai	6	3.35 ± 0.08		
Catechin	Changli	6	25.04 ± 0.28	10	0.000
	Penglai	6	26.95 ± 0.28		
Epicatechin	Changli	6	4.21 ± 0.58	10	0.000
	Penglai	6	7.97 ± 0.36		
Epigallo-catechin	Changli	6	14.67 ± 0.74	10	0.000
	Penglai	6	21.14 ± 0.54		

calcified soil of inclinable alkaline[14]. The chemical composition of different soils affected the formation of wine characteristics. The vine plant likes the element calcium and potassium, so adding lime or shell could increase the contents of the phenolic compounds, in other words, lime soils can make grapes accumulated higher phenolic compounds[15,16].

Phenolic compounds play an important part in the red wine production. However, these chemicals mainly drived from the skins and seeds of the grapes, including flavonoid and nonflavonoid, flavanol, anthocyanidin and a few leucoanthocyanidin. Polymerization, nonflavonoid and catechin form proanthocyanin were freedomly in the grapes, whereas in the wine, the compounds were also by the formation of combination, for example, they could combine with protein, making wine more and more steadily. At the same time, they could also combine with glucose to decrease the bitter, making wine gentler; anthocyanin and glucose could form the glycoside compounds. Anthocyanin was the main compounds of the wine colour, the existence of glycoside may improve water-solubility and chemical steady of proanthocyanin and phenolic compounds could increase the antioxidant ability of wine, consuming the oxygen, forming quinone and hydrogen peroxide[17-20].

Conclusion

The aim of the research was to determine the phenolic compounds of the different geographic origins in the grape berries. So the same cultivars from different growing areas and the different cultivars from the same areas were selected. Phenolic compounds were not only essential for the wine colour, but also important for the wine taste and astringency. These compounds were the main factors in forming the cultivar' state and specificity and also affected the aging processes of wine. The information presented in the current study should be informative in the development of wine quality with predictable characteristics for utilization in the commercial production of wine.

REFERENCES

1. J. Liu, Z. Du and H. Zhai, *J. Sino-Overseas Grapevine Wine*, **2**, 22 (2003).
2. A.K. Kanellis and K.A. Roubelakis-Angelakis, In eds.: G. Seymour, J. Taylor and G. Tucker, Grape, Biochemistry of Fruit Ripening, London: Chapman Epsilon Hall, p. 189 (1993).
3. R. Boulton, *Am. J. Enol. Vitic*., **52**, 67 (2001).
4. M.C. del Llaudy, R. Canals, J.M. Canals and F. Zamora, *Eur. Food Res. Technol*., **226**, 337 (2008).
5. S. Perez-Magarino and M.L. Gonzalez-San, *J. Food Chem*., **96**, 197 (2006).
6. E. Hebrero, C. Santos-Buelga and J.C. Rivas-Gonzalo, *Am. J. Enol. Vitic*., **39**, 227 (1988).
7. A. Ortega-Regules, I. Romero-Cascales, J.M. López-Roca, J.M. Ros-García and E. Gómez-Plaza, *J. Sci. Food Agric.*, **86**, 1460 (2006).
8. J.-M. Ryan and E. Revilla, *J. Agric. Food Chem*., **51**, 3372 (2003).
9. J.P. Roggero, S. Coen and B. Ragonnet, *Am. J. Enol. Vitic*., **37**, 77 (1986).
10. Z. Xinjie, Effects of Trellis System and Crop Load on Flavour Compounds in Winegrapes and Wines, D. Shandong Agricultural University (2005).
11. G. Mazza, L. Fukumoto, P. Delaquis, B. Girard and B. Ewert, *J. Agric. Food Chem*., **47**, 4009 (1999).
12. D. Kontkanen, A.G. Reynolds, M.A. Cliff and M. King, *Food Res. Int*., **38**, 417 (2005).
13. N. Mateus, S. Marques, A.C. Gonalves, J.M. Machado and V. de Freitas, *Am. J. EnoI. Vific*., **52**, 115 (2001).
14. X. Wang, X. Chen and J. Zhan, *J. Food Sci.*, **27**, 791 (2006) (In Chinese).
15. P. He, Grape Science: China Agriculture Press, Beijing, pp. 86-90 (1994).
16. K. Yokotsuka, K. Nagao, K. Nakazawa and M. Sato, *Am. J. Enol. Vitic*., **50**, 1 (1999).
17. M.S. Fernández-Pachón, D. Villaño, A.M. Troncoso and M.C. García-Parrilla, *Anal. Chim. Acta*, **563**, 101 (2006).
18. M.A. Rodriguez-delgado, G. Gonzlea-hemandez and J.E. Conde-gonzalez, *J. Food Chem.*, **78**, 523 (2002).
19. R.C. Minussi, M. Rossi, L. Bologna, L. Cordi, D. Rotilio and G.M. Pastore, *J. Food Chem*., **82**, 409 (2003).
20. S. Tinttunen and P. Lehtonen, *Eur. Food Res. Technol*., **212**, 390 (2001).